

The University of Chicago

CHLOROPHYLL PRODUCTION UNDER
VARIOUS ENVIRONMENTAL
CONDITIONS

A DISSERTATION SUBMITTED TO THE
FACULTY OF THE DIVISION OF THE BIO-
LOGICAL SCIENCES IN CANDIDACY FOR
THE DEGREE OF DOCTOR OF PHILOSOPHY

DEPARTMENT OF BOTANY

1934

By
GUS. B. ULVIN

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GUS B. ULVIN

(WITH EIGHT FIGURES)

Introduction

Chemical investigation of chlorophyll dates back to BERZELIUS, who, in 1838, treated the alcoholic extract of leaves with concentrated hydrochloric acid or alkali because he thought the leaf pigments were not decomposed by these reagents. He obtained only products of radical decomposition. Other investigators who used this same method were MULDER, 1844, and MOROT, 1849. It was believed by VERDEIL, 1851, that he could isolate pure chlorophyll by precipitating boiling alcoholic leaf extract with lime water and then treating the precipitate with hydrochloric acid. The hypothesis of the relationship between the coloring matter of leaves and of blood originated with VERDEIL (WILLSTÄTTER 11).

Different theories have naturally been entertained about the composition of chlorophyll. As late as 1906 some thought that monocotyledons and dicotyledons did not contain the same kind of chlorophyll. Others were able to find several different kinds of chlorophyll in a single plant, and an unlimited number when derived from different plant sources. By far the greatest contribution to the chemistry of chlorophyll up to the present time was made by WILLSTÄTTER (11) and his collaborators. Their work covers the period from 1906 to 1913.

Leaf powder was used exclusively in the older methods of chlorophyll extraction and in rather large portions. Twelve-liter bottles were used to obtain "bottle extracts," and percolators, ranging up to 25 liters in size, were sometimes used for extracting chlorophyll. Loss of phytol ($C_{20}H_{39}OH$) by alcoholysis, and change in chlorophyll on long standing, are disadvantages in these longer methods.

The extraction method as suggested by SCHERTZ (8) in 1928 and used in this experiment makes use of fresh leaf material instead of dry leaf powder as formerly used. Grinding and extracting requires one-half hour or a little longer, in contrast with 24 to 48 hours used just for extracting by some of the older methods.

The purpose of this investigation was to study the quantitative production of chlorophyll under different light periods in soy beans and radishes, and in corn in the presence or absence of iron or manganese individually and in the presence or absence of both in the nutrient solution. It was

¹ Contribution from the Hull Botanical Laboratory, the University of Chicago.

thought possible to control the carbohydrate manufacture within the plant by controlling the light, as against controlling the nitrogen supply in the nutrient solution, which is the method usually adopted when it is desired to produce plants with either a high or a low carbohydrate-nitrogen ratio.

Methods

All of the plants used in these experiments were grown in greenhouses covered with ordinary glass. In experiment I, soy beans of the variety Manchou, and two varieties of radishes, Early Carmine Turnip and French Breakfast, were used. After two plantings of Early Carmine Turnip no more seed of that variety was available and the other was substituted. One set of plants was grown in 10-hour light periods, the other in continuous light. The short light period plants were taken out of the dark room at 8 A.M. and put back at 6 P.M. The plants receiving continuous light received the total natural daylight, supplemented by electric light during the remaining hours of each 24. Three 1000-watt Mazda bulbs with reflectors were used 26.5 inches above each group of plants to supplement daylight.

The light intensity was measured several times and it was found that approximately 25 per cent. of the full intensity of sunlight was lost to the plants in the greenhouse. On a very bright day the intensity of full sunlight was found to be 8436 foot candles, 5536 foot candles in the greenhouse, and only 2046 foot candles under a curtain of muslin used to shade the plants on bright days. On a slightly hazy day, sunlight had an intensity of 6775 foot candles, but was reduced in the greenhouse to 5239 foot candles. In the first case the loss in passing through one thickness of greenhouse glass was 34.37 per cent., and in the second, 22.67 per cent. The loss indicated by two other readings was 31.13 per cent. The intensity from three 1000-watt electric light bulbs varied from 650 foot candles at a distance of 26.5 inches from the bulbs when all were new, to 431 foot candles after several months' use.

A hundred or more soy beans were seeded in a pot of sand under natural conditions, and when six or seven days old, seven plants were transplanted into each of several 2-gallon glazed pots. These plants were grown in quartz sand with DAVIDSON'S (1) nutrient solution 3 T, R, C₃. This is a four-salt solution supplying nitrogen as nitrate nitrogen in calcium nitrate and as ammonium nitrogen in ammonium sulphate. One-half molar stock solutions were made up of each of the four salts and these added in proper amounts to distilled water to make the nutrient solution. A few parts per million of manganese and boron were added to the nutrient solution at each application and iron as ferric phosphate whenever needed. The radish plants required much more Fe than did the soy beans. The soy bean plants

were planted at intervals and each individual planting is referred to as series 1, 2, etc.

In experiment II, a garden variety of sweet corn, Golden Bantam, was used. A three-salt nutrient solution containing nitrogen only as nitrate nitrogen in calcium nitrate was used. The effect of the presence or absence of iron and manganese on chlorophyll production was studied. A series of four treatments was used in which one set of four pots received neither iron nor manganese; the second set received Fe 1 p.p.m. but no manganese; the third set received Fe and Mn at the rate of 1 p.p.m. each; and the fourth set received Fe 15 p.p.m. and Mn 1 p.p.m.

CHLOROPHYLL EXTRACTION

The method of extracting and separating chlorophyll (α and β) from the other leaf pigments in green leaves, as suggested by SCHERTZ (8), was adopted with slight modifications for this experiment. Percentage chlorophyll content of leaves on both green weight and dry weight basis as well as on an area basis was wanted. Material was often scarce, therefore 5-gram leaf samples were used instead of 10-gram samples as recommended by SCHERTZ. Five-gram leaf samples were weighed out in duplicate and traced on paper as quickly as possible. One sample was placed in a refrigerator while the other sample was being ground and extracted according to the following method:

The 5-gram sample of fresh leaves, after being weighed and traced, was placed in a glass mortar with a little sodium carbonate to neutralize free acids, and ground for seven minutes in the dry mortar. Then about 25 grams of washed quartz sand were added and the grinding continued for seven minutes, when 30 cc. of pure acetone were added and the grinding continued for another seven minutes. A total of 21 minutes of grinding for each sample was found to be the shortest time in which the leaf tissue could be thoroughly disintegrated. This acetone extract was filtered through a Büchner funnel, under suction, and the residue washed with pure acetone until the filtrate was colorless. About 100 cc. were usually required for this. The residue was then washed with about 100 cc. of ether.

The combined ether-acetone filtrate was poured into a liter separatory funnel where the acetone was washed out with distilled water. Then 100-cc. portions of water were poured down the side of the separatory funnel with the aid of a small funnel. An aqueous layer, colored somewhat yellowish from the dissolved flavones, separated out below the ether. The liquids were rotated carefully and the aqueous layer drawn off. Repeating this washing three times with each sample of material used in this experiment was sufficient to make the last washing colorless; but with old apple leaves twelve or more washings were necessary.

The remainder of the flavones and anthocyanins, if present, were removed by washing the ether solution with a 1 per cent. solution of sodium carbonate. Some of the chlorophyll may be lost by this method; but if the flavones and anthocyanins are not removed, their presence results in too high a value for the chlorophylls when estimated colorimetrically. SCHERTZ (8) found that a 1 per cent. sodium carbonate solution would remove no chlorophyll from a pure chlorophyll-ether solution. He concludes that any chlorophyll removed by this method would be chlorophyll already decomposed in the leaf.

The ether solution was run into a 500-cc. bottle and 20 cc. of a colorless saturated potassium hydroxide-methyl alcohol solution added. This was shaken strongly and set in a refrigerator until the next day.

SEPARATION OF PIGMENTS

The ether-alkali-chlorophyll solution was taken from the refrigerator and poured into a liter separatory funnel, the bottle washed several times with a few cc. of distilled water and this added to the separatory funnel. One hundred cc. of ether should be added at this point, so the bottle in which the chlorophyll solution had been stored was washed with this ether. The separatory funnel was shaken strongly and allowed to stand for 20 minutes.

The chlorophyllin layer was then run off into a small separatory funnel. The ether in the large separatory funnel was washed two or three times with small quantities of water and this added to the small separatory funnel. The ether solution was washed with a few cc. of dilute potassium hydroxide solution, and again with water to remove remaining traces of chlorophyllin salts. Both washings were added to the small separatory funnel, the contents of which were now washed with 25-50 cc. of ether. The chlorophyllin layer was run into a 100-cc. volumetric flask, made up to the mark with water, and estimated with a colorimeter in comparison with GUTHRIE'S (4) chemical standard.

CHLOROPHYLL DETERMINATION

Quantitative determinations of chlorophyll have been made by different workers but each has used a method of his own. Recently attempts at standardization of the process have been made. SCHERTZ (9) recommends Lovibond color slides as a standard. Only the depth of color was compared here because the slides used did not give the same tint as the chlorophyll solution. That same year GUTHRIE (4) developed a chemical standard to be used in colorimetric determinations of chlorophyll. This standard is a solution which contains 28.5 cc. of copper sulphate solution ($\text{CuSO}_4 \cdot 5\text{H}_2\text{O}$, 10 grams per liter), plus 50 cc. of potassium dichromate solution ($\text{K}_2\text{Cr}_2\text{O}_7$,

20 grams per liter), plus 10 cc. of twice normal ammonium hydroxide, diluted to 100 cc. This solution is colorimetrically equivalent to a solution containing 85 mg. of chlorophyll per liter. GUTHRIE'S standard was adopted because it has advantages even over a solution of the pure pigment itself; it is more stable, more easily available, and exact duplications are easily made.

Experimentation

EXPERIMENT I

EFFECT OF SHORT LIGHT PERIOD AND CONTINUOUS LIGHT ON CHLOROPHYLL PRODUCTION

Radishes from which the data were obtained were seeded on three different dates, in both short and continuous light, and harvested at different ages. The Early Carmine Turnips were seeded December 26, 1931; the French Breakfast radishes on March 31 and May 17, 1932. Only one pot of radish plants, a pot of Early Carmine, seeded December 26, 1931, blossomed in the short light period. These blossomed when 146 days old. Of those grown in continuous light the average age for four pots at blooming was 36.8 days. The midrib of the radish leaves was removed before the leaves were used for chlorophyll determinations. At first whole plants were ground up in a Russwin mill and 10-gram portions used for analysis; but this method was soon abandoned for the blades of leaves so that chlorophyll content could be determined on the area basis also.

TABLE I

AGE AND VARIETY OF RADISH PLANTS, AND PERCENTAGE OF CHLOROPHYLL IN THE LEAVES ON GREEN AND DRY WEIGHT BASIS, AND ON AREA BASIS

VARIETY	AGE	SHORT LIGHT PERIOD			CONTINUOUS LIGHT		
		CHLORO- PHYLL GREEN WEIGHT	CHLORO- PHYLL DRY WEIGHT	Mg/cm ² .	CHLORO- PHYLL GREEN WEIGHT	CHLORO- PHYLL DRY WEIGHT	Mg/cm ² .
	<i>days</i>	<i>%</i>	<i>%</i>	<i>mg.</i>	<i>%</i>	<i>%</i>	<i>mg.</i>
Early Carmine	129	0.117	0.961				
Early Carmine	132	0.134	0.690	0.040			
Early Carmine	182	0.145	1.320	0.034			
French Breakfast	33	0.068	0.861		0.095	0.965	
French Breakfast	36	0.095	1.150	0.028	0.104	1.063	0.035
French Breakfast	40	0.107	0.970	0.024	0.097	0.51	0.026

The radish plants in the short light period did not blossom normally; the buds dropped off just before opening. In figure 1, the four plants on the right were grown in the short light period; the two in the center were



FIG. 1. Radish plants (see text for explanation).

FIG. 2. Soy bean plants 14 days old; short light period plants on left, continuous light plants on right.

FIG. 3. Soy bean plants 30 days old; short light period plants on left, continuous light plants on right.

FIG. 4. Soy bean plants 40 days old; short light period plants on left, continuous light plants on right.

FIG. 5. Soy bean plants 53 days old; same position as in figure 4.

FIG. 6. Soy bean plants all short light period: series 5, 40 days old; series 3, 53 days; series 2, 61 days old.

FIG. 7. Soy bean plants all continuous light plants: series 7, 14 days old; series 6, 30 days; series 5, 40 days; series 3, 53 days old.

FIG. 8. Soy bean plants: short light period plants on left, 80 days old; continuous light plant on right, 109 days old.

TABLE II
AGE OF RADISH PLANTS, PERCENTAGE OF CHLOROPHYLL IN LEAVES ON GREEN WEIGHT, DRY WEIGHT, AND AREA BASIS, AND PERCENTAGE DIFFERENCE IN CHLOROPHYLL CONTENT

AGE	SHORT LIGHT PERIOD			CONTINUOUS LIGHT			MORE OR LESS THAN IN SHORT PERIOD GREEN WEIGHT	MORE OR LESS THAN IN SHORT PERIOD DRY WEIGHT	MORE OR LESS THAN IN SHORT PERIOD PER CM ² .
	CHLORO-PHYLL GREEN WEIGHT	CHLORO-PHYLL DRY WEIGHT	Mg/cm ² .	CHLORO-PHYLL GREEN WEIGHT	CHLORO-PHYLL DRY WEIGHT	Mg/cm ² .			
days	%	%	mg.	%	%	mg.	%	%	%
33	0.068	0.861		0.095	0.965		+ 39.70	+ 12.07	
36	0.095	1.150	0.028	0.104	1.063	0.035	+ 9.47	- 8.18	+ 25.00
40	0.107	0.970	0.024	0.097	0.510	0.026	- 10.30	- 47.42	+ 8.33
129	0.117	0.961							
132	0.134	0.690	0.040						
182	0.145	1.320	0.034						

182 days old, and the two on the right were 40 days old. The two on the left were grown in continuous light and are also 40 days old. The four plants on the left are in about the same stage of blossoming, but with a difference of 142 days in age. The two plants in the center, however, grown in the short light period, had reached this stage 36 days before this photograph was taken, and advanced no further; the two on the left, grown

TABLE III

AGE OF RADISH PLANTS AT BLOSSOMING IN SHORT LIGHT AND CONTINUOUS LIGHT PERIODS

VARIETY	SEEDED	SHORT LIGHT PERIOD		CONTINUOUS LIGHT	
		BLOSSOMED	AGE	BLOSSOMED	AGE
			<i>days</i>		<i>days</i>
Early Carmine ...	Dec. 26, 1931	May 20, 1932	146	Feb. 5, 1932	42
French Breakfast	Mar. 4, 1932			Apr. 8, 1932	36
French Breakfast	Mar. 4, 1932			Apr. 13, 1932	41
French Breakfast	Mar. 31, 1932			May 2, 1932	33
French Breakfast	May 17, 1932			June 19, 1932	32
Average age			146		36.8

in continuous light, had just reached this same stage in flowering. The two plants in the center were grown in the same pot in the short light period; one produced a rather large radish while the other showed no enlargement whatever. However, a majority of the radish plants grown in the short period did produce radishes, while in the continuous light enlargement seldom occurred.

The soy beans were planted at irregular intervals in both light periods, and harvested at different ages. Seven plantings were made from March 10 to May 28, 1932, and each planting is called a series; there are seven series. These plants were harvested at different ages, varying in age from 18 to 110 days. A difference can be noticed in the plants in figure 2, seven days after being transplanted and placed in the different light periods. Short period plants are on the left and continuous light plants on the right. These plants were only 14 days old. A marked contrast is noted in figure 3, in which the plants are 30 days old.

The increasing difference with age when grown in short light and continuous light periods is emphasized in figures 4 and 5, 40 and 53 days old respectively. The plants in figure 6 were grown in the shorter period, and are, reading from left to right, 40, 53, and 61 days old. In this light period the plants did not grow beyond a definite size. If vegetative growth is continued it finds expression in numerous shoots from any or all of the nodes.

The opposite condition is found in plants grown in the long light period. Up to the time of harvesting the oldest plant there was apparently an increase in vegetative vigor with age. Figure 7 shows four pots of plants at different ages but all grown in continuous light. Not all the plants in figure 8 are of one age; the seven in the pot on the left grown in the shorter period are 80 days old, and the one plant in the other pot grown in continuous light is 109 days old. New leaves and branches can be seen appearing at the nodes of the plants on the left, grown in the 10-hour light period, while the lower 13 leaves have died and fallen off the plant grown in the 24-hour period.

A marked increase in dry weight per plant in the continuous light period over the other light period was found at all ages. While the percentage increase is consistent it is not in proportion to age. Some of the environmental factors, such as temperature and humidity, that were not controlled may have been responsible for the irregularity. Surely when relative humidity approaches the saturation point with the temperature ranging from 100° to 125° F., the life processes in soy bean plants are not normal.

The ratio between roots and tops was determined for 14 different ages of soy bean plants in both light periods. Seven plants were transplanted when six or seven days old into each of the 2-gallon glazed earthenware pots. One, two, and sometimes three pots of plants were used in each light period to secure sufficient material for the chlorophyll determinations. This accounts for the different numbers of plants indicated in table IV. Table V represents the results from 195 short period plants and from 125 continuous light plants. The ratios in the long light period show a rather sudden jump to 8.50 when 32 days old, with the highest value 9.58 at 37 days. The oldest plant, 110 days old when harvested, had a ratio of 8.82, but this figure may not be reliable. Seven plants had been planted in that pot, but one soon crowded the others so badly that six of them were removed by cutting off at the level of the sand to make room for the large one. When harvested, the roots may not have been separated accurately.

In the shorter period the ratio is more irregular but they also show a wider ratio with increasing age. EATON (3) found the largest root-top ratios in the longest light periods with soy bean plants 38 days old. The same was found to be the case in this experiment up to that age, but with no increase beyond 37 days up to 110 days in continuous light. There was a drop of one-half the ratio in one instance at 42 days. This latter figure might well be disregarded. In the short light period the root-top ratio continues to increase with age and reaches 16.77 for plants 71 days old. EATON's longest light exposure was only 3.5 hours longer than the short exposure used in this experiment.

TABLE IV
AGE OF SOY BEAN PLANTS, NUMBER, TOTAL DRY WEIGHT, DRY WEIGHT PER PLANT, AND PERCENTAGE INCREASE IN DRY MATTER PER PLANT IN CONTINUOUS LIGHT OVER SHORT LIGHT PERIOD

SERIES	AGE <i>days</i>	SHORT LIGHT PERIOD			CONTINUOUS LIGHT			PERCENTAGE IN- CREASE PER PLANT OVER SHORT PERIOD
		NUMBER OF PLANTS	TOTAL WEIGHT	WEIGHT PER PLANT	NUMBER OF PLANTS	TOTAL WEIGHT	WEIGHT PER PLANT	
6	18	21	<i>gm.</i> 4.09	<i>gm.</i> 0.194	14	<i>gm.</i> 4.43	<i>gm.</i> 0.316	% 62.89
4	25	21	9.29	0.442	14	7.95	0.567	28.28
7	25	21	8.27	0.393	14	10.01	0.715	81.93
2	28	14	4.55	0.325	7	5.41	0.772	137.53
5	28	14	8.34	0.595	14	15.93	1.137	89.11
3	31	9	4.70	0.522	7	12.67	1.810	246.76
5	32	14	10.60	0.757	7	10.91	1.558	105.81
4	34	7	6.10	0.871	7	10.23	1.461	67.78
4	37	7	5.39	0.770	7	20.19	2.884	274.54
6	42	14	19.91	1.422	12	33.48	2.790	96.22
1	55	7	6.70	0.957	7	26.38	3.768	293.73
5	59	18	53.21	2.956	7	66.10	9.442	219.41
3	66	14	48.44	3.460	7	74.95	10.707	209.45
2	71	14	47.85	3.42				
1	110				1	158.20	158.20	

TABLE V

AVERAGE DRY WEIGHT OF TOPS AND ROOTS OF SOY BEAN PLANTS IN SHORT LIGHT AND CONTINUOUS LIGHT PERIODS AND RATIO OF ROOTS TO TOPS

AGE	SHORT LIGHT PERIOD			CONTINUOUS LIGHT		
	TOPS	ROOTS	RATIO	TOPS	ROOTS	RATIO
	<i>gm.</i>	<i>gm.</i>		<i>gm.</i>	<i>gm.</i>	
18	0.144	0.050	2.88	0.216	0.100	2.16
25	0.251	0.190	1.32	0.444	0.125	3.55
25	0.334	0.059	5.66	0.586	0.128	4.57
28	0.438	0.157	2.78	1.005	0.132	7.61
28	0.271	0.054	5.01	0.678	0.094	7.21
31	0.477	0.444	1.07	1.581	0.228	6.93
32	0.642	0.114	5.63	1.394	0.164	8.50
34	0.685	0.185	3.70	1.261	0.200	6.30
37	0.740	0.107	6.91	2.598	0.271	9.58
42	1.243	0.178	6.98	2.223	0.483	4.60
55	0.857	0.100	8.57	5.490	0.600	9.15
59	2.703	0.252	10.72	8.542	0.900	9.49
66	3.202	0.271	11.81	9.642	1.064	9.06
71	3.225	0.192	16.77			
110				142.100	16.100	8.82

The age of the soy bean plants is included in tables IV, V, VI, VII, and VIII so that reference can easily be made from one table to another although not the same number of plants is included in each table. Only dry weight is used in table IV because it was thought that dry weight would be a more nearly accurate representation of the plants as a whole. Little can be said about fresh weight of roots except that some were wetter than others. That being the case the percentage of dry matter in the roots means little or nothing while the dry weight of the roots may be significant.

The largest percentage of increase per plant in continuous light over the shorter period was obtained in the plants harvested on May 4, when 55 days old. These were harvested before the temperature became abnormally high. There were no short light period plants of the same age as the oldest continuous light plant when harvested.

Chlorophyll content was determined on green weight, dry weight, and area basis. There was considerable variation in all three of these, as can be seen in table VI. On the green weight basis the leaves of the plants represented in this table usually show more chlorophyll in the long period than in the short period; 12 groups out of 13 show an increase varying from 5.41 to 53.92 per cent. The leaves showing more chlorophyll in the short light period were from plants harvested on June 19, after a few days of very hot weather, when the thermometer reached 114° F. on several

TABLE VI

AGE OF SOY BEAN PLANTS, CHLOROPHYLL CONTENT OF LEAVES ON GREEN WEIGHT, DRY WEIGHT, AND AREA BASIS, AND PERCENTAGE OF DIFFERENCE IN SHORT AND CONTINUOUS LIGHT PERIOD

Age	SHORT LIGHT PERIOD			CONTINUOUS LIGHT			MORE OR LESS THAN IN SHORT PERIOD DRY WEIGHT	MORE OR LESS THAN IN SHORT PERIOD DRY WEIGHT	MORE OR LESS THAN IN SHORT PERIOD DRY WEIGHT
	CHLORO-PHYLL GREEN WEIGHT BASIS	CHLORO-PHYLL DRY WEIGHT BASIS	CHLORO-PHYLL PER CM. ²	CHLORO-PHYLL GREEN WEIGHT BASIS	CHLORO-PHYLL DRY WEIGHT BASIS	CHLORO-PHYLL PER CM. ²			
days	%	%	mg.	%	%	mg.	%	%	%
18	0.195	1.090	0.031	0.281	1.780	0.034	+44.10	+63.30	+9.67
25	0.222	1.542	0.035	0.239	1.404	0.032	+7.65	-8.94	-8.57
25	0.203	1.290	0.024	0.214	1.040	0.028	+5.41	-19.37	+16.66
28	0.132	0.965		0.178	1.040		+34.84	+7.77	
28	0.204	1.641	0.032	0.314	1.848	0.039	+53.92	+12.61	+21.87
31	0.168	1.290	0.023	0.216	1.269	0.032	+28.57	-16.27	+39.13
32	0.225	1.451	0.030	0.263	1.435	0.024	+16.88	-1.10	-20.00
34	0.208	1.159	0.031	0.256	1.350	0.032	+23.07	+16.47	+3.22
37	0.225	1.180	0.037	0.256	1.340	0.035	+13.37	+13.56	-5.40
42	0.275	1.448	0.035	0.231	1.850	0.035	-16.00	+27.76	-0.00
55	0.238	1.192	0.034	0.256	1.340	0.037	+7.56	+12.41	+8.82
59	0.234	1.370	0.037	0.278	1.110	0.032	+18.80	-18.97	+1.33
66	0.234	1.064	0.035	0.279	1.111	0.035	+19.23	+4.41	-0.00
71	0.266	1.210	0.040						
110				0.230	0.838	0.033			

TABLE VII

AGE OF SOY BEAN PLANTS, NUMBER OF LEAVES PER 5-GRAM SAMPLE, SQUARE CM. PER LEAF, AND PERCENTAGE DRY MATTER IN LEAVES GROWN IN SHORT AND CONTINUOUS LIGHT PERIODS

AGE	SHORT LIGHT PERIOD				CONTINUOUS LIGHT			
	LEAVES PER 5- GRAM SAMPLE	AREA PER LEAF	AREA PER GRAM	DRY MATTER IN LEAVES	LEAVES PER 5- GRAM SAMPLE	AREA PER LEAF	AREA PER GRAM	DRY MATTER IN LEAVES
<i>days</i>		<i>cm.²</i>	<i>cm.²</i>	<i>%</i>		<i>cm.²</i>	<i>cm.²</i>	<i>%</i>
18 ...	39.00	8.00	62.40	17.85	37.00	11.08	82.00	15.70
25 ...	35.00	9.45	66.20	14.39	26.50	13.98	74.12	17.00
25 ...	38.00	11.10	84.40	15.71	31.50	11.86	74.75	20.60
28 ...	24.00	13.37	64.21	12.43	16.00	25.14	80.45	17.00
31 ...	27.50	13.11	77.29	13.00	15.00	22.11	83.19	17.00
32 ...	24.00	15.37	73.78	15.50	15.00	30.02	90.06	18.50
34 ...	24.50	13.72	67.24	16.66	19.50	20.35	79.40	18.84
37 ...	21.50	14.14	60.84	17.00	10.50	34.38	72.20	19.00
42 ...	15.50	24.82	76.96	19.00	10.75	30.58	65.74	12.50
55 ...	19.50	14.87	58.00	20.00	5.00	68.20	68.20	19.00
59 ...	9.75	31.97	62.35	17.00	6.12	69.54	85.19	25.00
66 ...	13.50	24.54	66.28	22.00	5.06	77.29	78.22	25.00
71 ...	14.00	22.67	65.29	22.00				
110 ...					2.85	121.05	69.00	27.50
Aver- age...	23.51	16.54	68.09	17.11	15.44	41.19	79.96	19.43

occasions. During such weather the plants were watered three times a day and the floor sprinkled five or six times.

The size of the soy bean leaves varies with the age of the plants. They vary in size from 8 to 31.97 cm.^2 in the short light period but in continuous light from 11.08 to 121.05 cm.^2 In other words, the number of leaves per 5-gram sample of fresh weight varies from 39.00 to 9.75 leaves in the former and from 37 to 2.85 leaves in the latter, the larger leaves occurring on the older plants in both light periods (table VII).

In an attempt to determine the efficiency of chlorophyll in the two light periods, the ratio of the chlorophyll in the total leaves of each plant to the total dry weight of each plant respectively was calculated and these differences indicated in table VIII as greater or less than the ratios found for plants of like age in the short light period. The result was not at all striking. The ratios in the shorter period varied from 126 to 284 and in continuous light from 117 to 283. In six of the 13 comparisons the ratio was smaller in the continuous exposure; in the remaining seven the ratio was larger.

TABLE VIII

AGE OF SOY BEAN PLANTS, TOTAL DRY WEIGHT, TOTAL CHLOROPHYLL IN LEAVES, RATIO OF CHLOROPHYLL TO DRY MATTER, AND DIFFERENCE BETWEEN THIS RATIO IN SHORT LIGHT PERIOD AND CONTINUOUS LIGHT

AGE	SHORT LIGHT PERIOD.			CONTINUOUS LIGHT			DIFFERENCE IN RATIO, ABOVE OR BELOW SHORT LIGHT PERIOD
	TOTAL DRY WEIGHT	TOTAL CHLOROPHYLL IN LEAVES	RATIO CHLOROPHYLL TO DRY MATTER	TOTAL DRY WEIGHT	TOTAL CHLOROPHYLL IN LEAVES	RATIO CHLOROPHYLL TO DRY MATTER	
<i>days</i>	<i>gm.</i>	<i>gm.</i>		<i>gm.</i>	<i>gm.</i>		
18	4.09	0.0179	228	4.43	0.0337	131	- 97
25	9.29	0.0515	180	7.95	0.0385	206	+ 26
25	8.27	0.0486	170	10.01	0.0449	223	+ 53
28	4.55	0.0247	184	5.41	0.0256	211	+ 27
28	8.34	0.0598	139	15.93	0.1346	118	- 21
31	4.70	0.0337	139	12.67	0.0675	187	+ 48
32	10.60	0.0812	130	10.91	0.0692	157	+ 27
34	6.10	0.0353	172	10.23	0.0608	168	- 4
37	5.39	0.0423	126	20.19	0.1159	174	+ 48
42	19.91	0.1354	147	33.48	0.1411	237	+ 90
55	6.70	0.0358	187	26.38	0.2250	117	- 70
59	53.21	0.2270	234	66.10	0.3336	198	- 36
66	48.44	0.1960	247	74.95	0.3292	227	- 20
71	47.85	0.1684	284				
110				158.20	0.5584	283	
Average...			183			188	+ 5

TABLE IX

AGE OF MANCHU SOY BEAN PLANTS WHEN FIRST PODS APPEARED IN THE SHORT LIGHT PERIOD

SERIES	SHORT LIGHT PERIOD		
	SEEDED (1932)	POD APPEARED (1932)	AGE
			<i>days</i>
1	Mar. 10	Apr. 20	42
2	Apr. 8	May 20	43
2	Apr. 8	May 21	44
3	Apr. 16	May 29	44
5	Apr. 29	June 11	44
Average.....			43.4

TABLE X

PERCENTAGE OF NITRATE NITROGEN, TOTAL NITROGEN, REDUCING AND NON-REDUCING SUGARS, STARCH, AND TOTAL WATER AND ALCOHOL-SOLUBLE CARBOHYDRATES IN STEMS AND PETIOLES OF SOY BEAN PLANTS

SOY BEAN PLANTS	NO ₃ NITRO-GEN	TOTAL NITRO-GEN	REDUC-ING SUGAR	NON-RE-DUCING SUGAR	STARCH	TOTAL WATER AND ALCOHOL-SOLUBLE CARBOHYDRATES
	%	%	%	%	%	%
Short light period, 25-28 days old	0.37	8.06	1.03	0.04	1.22	2.29
Continuous light, 25-28 days old	0.17	5.55	1.06	0.10	1.40	2.56
Short light, 42 days old	0.32	6.22	1.70	0.46	1.65	3.81
Continuous light, 42 days old	0.13	5.17	2.23	0.38	0.56	3.17

The results shown in table X seem to indicate that nitrate nitrogen was stored in the short light period plants and that the percentage of total nitrogen was also greater in these plants. Carbohydrates may have been the limiting factor in the shorter period which was caused by the limited exposure to light.

EXPERIMENT II

EFFECT OF IRON AND MANGANESE ON CHLOROPHYLL PRODUCTION
IN YOUNG CORN PLANTS

Zea mays saccharata, the garden variety Golden Bantam, was used in this experiment. The plants on which chlorophyll determinations were made were harvested June 16, when 34 days old. One pot in each treatment was harvested. Sixteen days later, when it appeared that the plants were becoming crowded, two more pots in each treatment were harvested; only fresh and dry weight determinations were made on these. The gain in green weight and dry weight on the addition of both Fe and Mn, or of Fe or Mn added separately, compared with the condition when both were absent, can be considered.

Four separate treatments were used: the plants in treatment A received neither iron nor manganese; those in B received 1 p.p.m. of Fe; those in C received 1 p.p.m. of both Fe and Mn; and those in D received 15 p.p.m. Fe and 1 p.p.m. Mn in the nutrient solution applied by the drip culture method.

TABLE XI

CHLOROPHYLL PRODUCTION IN YOUNG CORN PLANTS WITH PARTS PER MILLION OF IRON AND MANGANESE IN THE NUTRIENT SOLUTION

	A	B	C	D
P.P.M. Fe	0	1	1	15
P.P.M. MN	0	0	1	1
	<i>gm.</i>	<i>gm.</i>	<i>gm.</i>	<i>gm.</i>
Weight of chlorophyll in 5 grams of fresh leaves.....	0.0077	0.0095	0.0100	0.0127
	%	%	%	%
Percentage of chlorophyll in leaves	0.1530	0.1906	0.2000	0.2612
Percentage increase in chlorophyll over A		24.5751	30.7189	70.7189
Percentage increase in chlorophyll over B			4.9317	37.0409
Percentage increase in chlorophyll over C				30.6000
	<i>cm.²</i>	<i>cm.²</i>	<i>cm.²</i>	<i>cm.²</i>
Cm. ² per 5-gram sample of fresh leaves	352.1000	371.2000	388.2000	415.8000
Chlorophyll, mg./cm. ²	0.0218	0.0255	0.0257	0.0305
	%	%	%	%
Percentage of dry matter in leaves	11.5000	12.0000	14.0000	14.5000

An experiment on nitrogen assimilation by sugar-cane and sweet corn plants at different soil temperatures (6) was being carried on at the same time, and just prior to harvesting the sugar-cane plants, chlorophyll determinations were made on some of the leaf-blade material. These sugar-cane plants were grown in the same type of quartz sand as was used for the soy bean, radish, and corn plants. But the pots were placed in automatically controlled, electrically heated water tanks to provide soil temperatures of 15°, 25°, and 30° C. Tanks 1 and 2 were to be kept at 15° but for a time during August this temperature was exceeded. Tanks 3 and 4 were kept at a temperature of 25° throughout the experiment, and tanks 5 and 6 at 30° C. The plants in tanks 1, 3, and 5 received their nitrogen as $(\text{NH}_4)_2\text{SO}_4$ and NH_4OH , while those in tanks 2, 4, and 6 received their nitrogen as $\text{Ca}(\text{NO}_3)_2$. Both nutrient solutions had a reaction value of pH 7.

The leaves used for chlorophyll analyses were in every instance the third from the top of the plants.

TABLE XII

AVERAGE FRESH WEIGHT PER CORN PLANT UNDER DIFFERENT TREATMENTS WITH Fe AND Mn,
GAIN IN WEIGHT PER PLANT AND PERCENTAGE OF GAIN OVER PLANTS
NOT RECEIVING Fe AND Mn

	A		B		C		D	
P.P.M. Fe	0		1		1		15	
P.P.M. Mn	0		0		1		1	
	NUM- BER OF PLANTS	GREEN WEIGHT	NUM- BER OF PLANTS	GREEN WEIGHT	NUM- BER OF PLANTS	GREEN WEIGHT	NUM- BER OF PLANTS	GREEN WEIGHT
34 days old	14	<i>gm.</i> 10.90	16	<i>gm.</i> 15.43	17	<i>gm.</i> 22.42	13	<i>gm.</i> 23.19
Percentage gain over A				% 41.55		% 105.68		% 112.75
50 days old	31	<i>gm.</i> 39.49	27	<i>gm.</i> 55.76	29	<i>gm.</i> 54.71	28	<i>gm.</i> 58.49
Percentage gain over A				% 41.20		% 38.54		% 48.11

TABLE XIII

DRY WEIGHT PER CORN PLANT UNDER DIFFERENT TREATMENTS
WITH Fe AND Mn

	A		B		C		D	
P.P.M. Fe	0		1		1		15	
P.P.M. Mn	0		0		1		1	
AGE	NUM- BER OF PLANTS	DRY WEIGHT	NUM- BER OF PLANTS	DRY WEIGHT	NUM- BER OF PLANTS	DRY WEIGHT	NUM- BER OF PLANTS	DRY WEIGHT
34	14	<i>gm.</i> 1.75	16	<i>gm.</i> 1.05	17	<i>gm.</i> 1.54	13	<i>gm.</i> 1.75
50	31	3.27	27	4.85	29	4.80	28	5.71
Gain in weight in 16 days ..		2.52		% 3.80		% 3.26		% 3.96
Percentage gain in 16 days over A				50.79		29.36		57.14

TABLE XIV

PERCENTAGE OF CHLOROPHYLL IN SUGAR-CANE LEAVES ON GREEN AND DRY WEIGHT BASIS,
CHLOROPHYLL PER SQUARE CM., AND PERCENTAGE OF DRY MATTER (6)

TANK	PERCENTAGE OF CHLOROPHYLL		Mg./CM. ²	DRY MATTER	PROPORTION OF CHLOROPHYLL	
	GREEN WEIGHT	DRY WEIGHT			GREEN WEIGHT BASIS	AREA BASIS
	%	%	mg.	%	%	%
1	0.220	0.903	0.0389	24.4	100.00	100.00
2	0.263	1.196	0.0438	22.0	119.52	112.59
3	0.223	1.016	0.0420	22.0	101.36	107.96
4	0.252	1.031	0.0500	24.4	114.54	128.53
5	0.202	0.886	0.0379	22.8	91.81	97.42
6	0.204	0.825	0.0372	24.8	92.72	95.63

Discussion

The amount of chlorophyll contained in leaves necessarily limits the amount of photosynthesis under conditions as they normally exist in nature. As early as 1879, WEBER (MILLER 7) noticed that equal areas of leaves of different plants grown under similar conditions had different assimilatory powers. In 1882 HABERLANDT attempted to explain this by counting the chloroplasts in a unit area, but MILLER concludes that since there is no evidence that all chloroplasts contain the same amounts of chlorophyll no definite conclusions can be drawn from this method.

It was found by LUBIMENKO (5) that shade plants contained more chlorophyll than sun plants, and could accomplish the same amount of photosynthesis with less illumination than sun plants. He noted that if plants are grown under lowland and alpine conditions, the lowland plants may have as much as 2.3 times more chlorophyll than the alpine plants of the same species. Here there is a marked difference in the light as well as in other growth conditions. If light were the only variable factor in the environment, what differences in chlorophyll production would be found in the same species of plants?

Soy bean and radish plants were grown with this question in mind. The method adopted was to grow one set of plants in daylight from 8 A.M. to 6 P.M., supplemented by artificial light on cloudy days, and the other set in the total natural daylight supplemented as described, plus artificial light the remaining hours of the 24. During the winter season, both sets of plants received the same amount of daylight, but as the days became longer

the continuous light plants received more and more daylight in proportion to the amount of artificial light received. It would seem that this condition might be favorable to the continuous light plants.

The soy bean, a so-called short-day plant, and the radish, a long-day plant, were chosen because they were contrasting plants so far as light requirements for seed production were concerned. Under continuous light, radish plants produced flowers in from 32 to 42 days, with an average of 36.8 days. Only one pot of radishes was grown long enough in the short light period for flower production. These were planted December 26, 1931, and blossomed May 20, 1932, 146 days later. The radishes planted on the same date in continuous light blossomed February 5, 1932, when 42 days old. Temperature or light may be a factor here.

A negative correlation seems to exist between temperature and the age of radish plants at blossoming. Those seeded December 26, 1931, and grown in continuous light blossomed in 42 days, while those seeded May 17, 1932, and grown in continuous light blossomed in 32 days, a difference of more than 31 per cent. of the time required in late spring. Growth form varied considerably in both light periods, especially in the short light period. This may be noticed in figure 1.

Length of exposure to light seemed to have the opposite effect on soy bean plants. In no case were opened flowers seen on the plants, so the age used here is that at which pods were first seen in the short exposure. The range was very narrow, 41 to 44 days as compared with 32 to 42 days for radishes in continuous exposure. No pods occurred on any soy bean plant grown in continuous light although one plant was harvested when 110 days old.

The vegetative vigor of soy bean plants seemed to increase with age in continuous light. The leaves became enormous in size; only 2.85 leaves were required for 5-gram samples of fresh leaves for chlorophyll analysis. These averaged 121.05 cm.² per leaf. Bean plants of series 3 were harvested in both light periods when 66 days old, and 13.50 leaves with an average area of 24.54 cm.² were required for 5-gram samples of fresh leaf material in the short exposure, while only 5.06 leaves with an average area of 77.29 cm.² were required in the continuous exposure. The leaves produced in continuous light have a surface area more than three times as great as those produced in the short exposure. At 32 and 18 days the figures are 24 leaves with areas of 15.37 cm.², 15 leaves with areas of 30.02 cm.², and 39 and 37 leaves with areas of 8.00 and 11.08 cm.² respectively (table VII).

The thickness of the soy bean leaves was not determined, but the fourth and eighth columns of table VII indicate an appreciable difference. One gram of the fresh leaf blade material in the continuous light for 13 dupli-

cate measurements of 5 grams each averages 79.96 cm.², and in the short exposure only 68.09 cm.² This average difference of 11.87 cm.² of area per gram shows that the leaves were thinner in the continuous light. The leaves in the short exposure felt more leathery to the touch than the others, but the greater length and also possibly a greater number of leaf hairs in the long exposure may have accounted for that.

Only the leaves were analyzed for chlorophyll content. At an early age the plants in continuous light would naturally be expected to contain more chlorophyll than those of a corresponding age in the shorter light exposure. However, two analyses were made of 25-day old plants that had more chlorophyll in the short light period. There is considerable variation in the total chlorophyll content of the leaves as can be seen from the data of table VIII. On the green weight basis, for 13 duplicate determinations in both light periods, the average chlorophyll content was 19.83 per cent. more in continuous light; on the dry weight basis 7.20 per cent. more; and on the area basis ~~6.67~~^{5.85} per cent. more than in the short exposure. Radish leaves showed an average of 12.95 per cent. more chlorophyll in continuous light on the green weight basis; 14.53 per cent. less on dry weight basis; but 16.66 per cent. more on the area basis (calculated from data of tables I and II).

An attempt was made to discover whether there is any correlation between the total dry weight and the chlorophyll content of the leaves of soy bean plants by dividing the dry weight by the amount of chlorophyll in the total leaves, assuming the remaining leaves contained the same amount of chlorophyll as the 5-gram samples used in analysis (table VIII). A high ratio would indicate greater efficiency than a lower ratio, other things being equal in the two periods; but things were not equal. There are many possibilities for errors. The greatest single error might easily be due to the fact that in the continuous exposure many of the lower leaves dropped off before analyses were made; consequently an indeterminable amount of chlorophyll was lost. No leaves dropped off the plants in short exposure, so that a more nearly accurate value for chlorophyll was obtained.

In the plants analyzed for chlorophyll when 18 days old, corresponding leaves (*i.e.*, the first two foliage leaves and one compound leaf) were gathered from each plant. Leaves from 14 plants were used in the continuous light experiments and from 21 plants in the 10-hour day experiments. The ratios of chlorophyll to dry matter in this instance are 1:228 and 1:131 in the short and continuous exposures respectively. There is an increase of chlorophyll in the total leaves in continuous light of 87.87 per cent., and the 14 plants in this set produced 8.31 per cent. more dry weight than did the other 21. But when this increase is placed on a "per plant" basis, the chlorophyll increase is 182 per cent., and the dry weight increase (table IV)

is 62.89 per cent. per plant. In view of the fact that growth conditions in each light period were maintained as nearly identical as possible, these irregularities are striking.

Cross-sections of soy bean leaves were examined under a microscope and it was thought a difference in arrangement of the chloroplasts could be noticed. The chloroplasts were more or less plano-convex, and in the continuous light these were arranged more regularly, with their flat sides toward the cell wall.

In experiment II with corn a different approach to the problem of chlorophyll production was attempted. It is conceded that chlorophyll is not formed in the absence of iron and that only very small quantities are required. The exact function of iron in chlorophyll production is not known since iron does not occur in the chlorophyll molecule. MILLER (7) cites ODDO and POLLACCI as stating that *Zea mays*, *Solanum nigrum*, *Datura stramonium*, *Euphorbia* sp., and *Aster sinensis* were grown in a nutrient solution in which Fe was replaced by the magnesium salt of pyrrole-carbonic acid. These investigators think that Fe has a catalytic action on the formation of the pyrrole nucleus which is the center of the chlorophyll complex. If the pyrrole nucleus is already present they think that Fe is not necessary for the formation of chlorophyll.

WILLSTÄTTER's observations seem to support this theory, but DEUBER (2) could not confirm it. He grew corn, cow pea, soy bean, and *Spirodela* in nutrient solutions containing different proportions of a magnesium salt of pyrrole-carbonic acid substituted for iron, but in no case did this compound prevent chlorosis of the leaves of the plants.

Corn plants were grown in no. 3 quartz sand of the following composition: SiO_2 , 98.95 per cent.; Al_2O_3 , 0.6 per cent.; iron as Fe_2O_3 , 0.31 per cent.; and no calcium nor magnesium. A nutrient solution made up of chemically pure salts was used with this sand in four different treatments as previously described. In A, the culture not receiving iron in the nutrient solution, 5-gram duplicate samples of fresh leaves were found to contain 0.1530 per cent. chlorophyll (table XI). In B, with the addition of Fe 1 p.p.m., a chlorophyll content of 0.1906 was found, a 24.75 per cent. increase over A. With the addition of Fe and Mn each 1 p.p.m., a very small increase in chlorophyll content was obtained in C; but when Fe 15 p.p.m. and Mn 1 p.p.m. were added to culture D a 30.6 per cent. increase over C was obtained. This makes a total increase of 70.71 per cent. over culture A.

The effect of Mn 1 p.p.m. with no Fe, or Mn 15 p.p.m. and Fe 1 p.p.m. in contrast to cultures B and D, was not determined; but the data indicate that iron was more effective, under the conditions of this experiment, than manganese in stimulation of chlorophyll production.

In experiment II there is a gradual increase in dry weight with the increase in chlorophyll content. The thickness of the leaves decreases with the increase in chlorophyll content per cm.² The increase in dry matter, the increase in chlorophyll content on the green weight basis, and the increase in the number of cm.² per gram of fresh leaf material are positively correlated in this experiment.

An increase in chlorophyll production in corn up to 67 per cent. over the check was obtained by SHULL and MITCHELL (10) with the use of x-ray treatments. The seeds were treated for 1, 2, 3, 4, and 5 minutes through a 1-mm. aluminum filter. The greatest increase occurred in the 2-minute treatment but all treatments showed an increase over the control.

In the experiment on the assimilation of nitrogen as NO₃ or as NH₃ by sugar-cane at soil temperatures of 15°, 25°, and 30° C., a marked difference in chlorophyll content was observed. Considering the chlorophyll content of the plants receiving nitrogen as NH₃ in the 15° C. tank as 100 per cent., the most chlorophyll was found in the NO₃ treatment at 25° C. soil temperature on the area basis. At all three soil temperatures more chlorophyll was found in the nitrate nitrogen treated plants than in those receiving their nitrogen as ammonium nitrogen. The highest percentage of chlorophyll on both the green weight and the dry weight basis occurred in the NO₃ treated plants at a soil temperature of 15° C. (table XIV).

Summary

1. Radish plants continued vegetative growth in a 10-hour day for 182 days and were still vigorous when harvested. Those in continuous light blossomed in about 36 days, soon ripened seed, and died. Radish plants in the short light period tended to store food while those in continuous light seldom produced enlarged storage organs.

2. Size of leaves in the soy bean increased with age in both light periods, but much more rapidly in continuous light. When 66 days old the leaves in continuous light were more than three times as large as those of the same age in the 10-hour period.

3. The leaves tend to be thinner with continuous light exposure, although this difference may not be significant.

4. On the average, leaves developed in continuous light contain more chlorophyll on the green weight, dry weight, and area basis than do those developed in a short light period.

5. Vegetative activity in soy beans seemed to increase with age in continuous light exposure; the reverse was the case with a short exposure.

6. Chlorophyll production was markedly stimulated in corn plants by the addition of Fe 1 p.p.m. and 15 p.p.m. to the nutrient solution.

7. Little response was obtained with Mn 1 p.p.m. Impurities in the sand may have been the limiting factor.

8. The percentage of dry matter of corn leaves increased in inverse proportion to the thickness of the leaves but in direct proportion to the chlorophyll content on green weight, dry weight, and area basis.

9. Gains in chlorophyll content are reported in corn plants as a result of x-ray treatments.

10. Under conditions of a nitrogen assimilation experiment with sugar-cane, more chlorophyll was produced by the plants receiving nitrogen as NO_3 than by those receiving nitrogen as NH_3 in each of three soil temperatures.

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LITERATURE CITED

1. DAVIDSON, O. W. An application of the triangle system in determining a nutrient solution suitable for research with the peach in sand culture. *Proc. Amer. Soc. Hort. Sci.* **25**: 354-358. 1928.
2. DEUBER, C. G. Can a pyrrole derivative be substituted for Fe in the growth of plants. *Amer. Jour. Bot.* **13**: 276-285. 1926.
3. EATON, S. V. Effects of variation in day-length and clipping of plants on nodule development and growth of soy bean. *Bot. Gaz.* **91**: 113-143. 1931.
4. GUTHRIE, J. D. A stable colorimetric standard for chlorophyll. *Amer. Jour. Bot.* **15**: 86-87. 1928.
5. LUBIMENKO, W. Cited by MILLER (7, page 465).
6. MARTIN, J. P., and ULVIN, G. B. Nitrogen assimilation as nitrate nitrogen and ammonium nitrogen by *Zea mays saccharata* and sugar-cane plants at different soil temperatures. 1932 (unpublished).
7. MILLER, EDWIN C. *Plant Physiology*. New York. 1931.
8. SCHERTZ, F. M. The extraction and separation of chlorophyll (α and β), carotin and xanthophyll in fresh green leaves, preliminary to their quantitative determination. *Plant Physiol.* **3**: 211-216. 1928.
9. ———. The quantitative determination of chlorophyll. *Plant Physiol.* **3**: 323-334. 1928.
10. SHULL, C. A., and MITCHELL, J. W. Stimulative effects of x-rays on plant growth. *Plant Physiol.* **8**: 287-296. 1933.
11. WILLSTÄTTER, R., and STOLL, A. *Investigations on chlorophyll*. SCHERTZ and MERZ, authorized translation. 1928.

